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CYP1A1 and *CYP1B1* genotypes, haplotypes, and TCDD-induced gene expression in subjects from Seveso, Italy

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Abstract

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is highly toxic in experimental animals, and is known to induce cytochrome P450 (*CYP*) gene expression. We investigated the effect of *CYP1A1* and *CYP1B1* variant genotypes and haplotypes on *CYP1A1* and *CYP1B1* mRNA expression and ethoxyresorufin-*O*-deethylase (EROD) activity in lymphocytes from 121 subjects from the Seveso population, Italy, accidentally exposed to TCDD in 1976. The 3′UTR 3801T>C and I462V variants of *CYP1A1* were present in 16% and 6% of the subjects, respectively. The frequency of *CYP1B1* variants was 85.2% for L432V, 49.6% for R48G and A119S, and 28.7% for N453S. There was complete linkage disequilibrium (LD) among the *CYP1B1* variant loci ($D' = -1$) and high LD among the *CYP1A1* loci ($D' = 0.86$). Gene expression measured by RT-PCR did not vary by *CYP1B1* genotype in uncultured lymphocytes. However, when lymphocytes were treated in vitro with 10 nM TCDD, *CYP1B1* and *CYP1A1* mRNA expression was strongly induced and modified by *CYP* variant alleles. Specifically, the *CYP1B1*3* haplotype (L432V) was associated with increased *CYP1B1* mRNA expression ($P = 0.03$), following an additive model; the *CYP1A1* I462V

Abbreviations: EROD, ethoxyresorufin-O-deethylase; TCDD, 2,3,7,8-tetrachlorodibenzo-p-dioxin; CYP, cytochrome P450; LD, linkage disequilibrium; AhR, aryl hydrocarbon receptor; SNP, single nucleotide polymorphisms; ppt, parts per trillion; RT-PCR, reverse transcription polymerase chain reaction; CI, confidence interval; IQR, inter-quartile range; EM-algorithm, expectation-maximization algorithm; SAM, single-point additive model

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polymorphism was positively, although not significantly, associated with *CYP1A1* expression. The *CYP1B1*3* variant may have affected *CYP1B1* expression in subjects highly and acutely exposed to dioxin at the time of the accident. Although based on small number of subjects, a slight increase in eczema ($P = 0.05$, $n = 8$) and urticaria ($P = 0.02$, $n = 2$) was observed 20 years after the accident in subjects carrying the *CYP1B1*3* allele. Genetic variation in cytochrome P450 induction may identify subjects with variable responsiveness to TCDD and potentially increased risk of disease.

Keywords: 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin; *CYP1A1*; *CYP1B1*; Genotypes; Haplotypes; Gene expression

1. Introduction

In 1976, an industrial accident exposed several thousand people to substantial quantities of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in Seveso, Italy. TCDD is carcinogenic and results in severe developmental, endocrinological, immunological, and reproductive toxicity in experimental animals (Birnbaum and Tuomisto, 2000; Dragan and Schrenk, 2000). A significant increase in cancer incidence of (Bertazzi et al., 1993; Pesatori et al., 1999) and mortality from (Bertazzi et al., 2001) lymphohematopoietic neoplasms has been reported in the contaminated area. Approximately 20 years after the exposure, we randomly sampled 121 subjects, of whom 62 were from the most exposed zones (A and B) and 59 were from the surrounding non-contaminated area, and measured plasma TCDD levels and biomarkers of possible TCDD toxicity. High TCDD plasma levels (ranging from 1.0 to 89.9 ng/kg lipid or part per trillion) were still present in the exposed individuals after a period of time roughly equivalent to two biological half-lives, with significantly higher levels in women (Landi et al., 1997, 1998). In this population, we found a negative association between TCDD plasma levels and both aryl hydrocarbon receptor (AhR) expression in uncultured cells and ethoxyresorufin-*O*-deethylase (EROD) activity in cultured cells (Landi et al., 2003).

We hypothesized that the lack of association between TCDD plasma levels and *CYP1A1* and *CYP1B1* mRNA expression could reflect inter-individual variation in inducibility resulting from polymorphisms in the same genes. Genetic and biochemical studies indicate that the AhR is necessary for most of the toxic effects of TCDD (Puga et al., 2000; Safe, 1986). Following TCDD exposure, AhR acts as a ligand-activated receptor and transcription factor that forms an active heterodimer with the aromatic hydrocarbon nuclear translocator (ARNT/HIF-1β) and activates the transcription of xenobiotic metabolizing enzymes, such as cytochrome P4501A1 (*CYP1A1*) and P4501B1 (*CYP1B1*) as well as other genes (Hoffman et al., 1991; Jiang et al., 1996; Whitlock, 1999). *CYP* genes are polymorphic, and many of these polymorphisms result in enzymes with altered metabolic activity (Ingelman-Sundberg, 2002). To verify whether the lack of association between TCDD plasma levels and *CYP* gene expression could be the result of differential TCDD-related inducibility in subjects carrying variant alleles of *CYP* genes, we measured the frequency of variant alleles of the *CYP1A1* and *CYP1B1* genes, and their association with *CYP* gene expression and TCDD-related inducibility in lymphocytes from the Seveso subjects.

Previous work by Stoilov and collaborators (Bejjani et al., 2000; Stoilov et al., 1998) and Aklillu and collaborators (Aklillu et al., 2002) have identified at least nine polymorphic sites in the *CYP1B1* gene (2p22-p21, OMIM# 601771). Four polymorphisms, which cause amino acid substitutions (Aklillu et al., 2002; Bailey et al., 1998; Stoilov et al., 1998), have been examined for their potential role in cancer risk: Arg to Gly at codon 48, Ala to Ser at codon 119, Leu to Val at codon 432, and Asn to Ser at codon 453. *CYP1B1* variants have been associated with various cancers, such as ovarian (Goodman et al., 2001), breast (Watanabe et al., 2000), lung (Watanabe et al., 2000), colorectal (Fritsche et al., 1999), head and neck (Ko et al., 2001), prostate (Tanaka et al., 2002), and endometrial cancer (Sasaki et al., 2003).

Several single nucleotide polymorphisms (SNPs) in the *CYP1A1* gene (15q22.2-q22.4, OMIM# 108330) have been identified (Cascorbi et al., 1996), two of which have been associated with cancer risk: the first (Spurr et al., 1987) is a thymine/cytosine polymorphism in the 3′UTR region; the second is an adenosine/guanosine polymorphism, at nucleotide 2455,

which results in an isoleucine/valine substitution in the heme-binding region in exon 7, codon 462 (I462V). These polymorphisms have been correlated with an increased risk of lung cancer, particularly in non-smokers, in women, and at younger age, but not all such studies agree (Houlston, 2000; Hung et al., 2003; Kiyohara et al., 1998; Le Marchand et al., 2003; Taioli et al., 2003).

We report here the results of the association between *CYP1B1* genotypes and haplotypes and *CYP1B1* constitutive and induced mRNA expression in uncultured, mitogen-treated, and in mitogen-treated lymphocytes cultured in vitro with 10 nM TCDD (defined here as TCDD/mitogen-treated lymphocytes), from the Seveso subjects. We also report results of the analysis of *CYP1A1* genotypes and their association with *CYP1A1* expression and inducibility, as well as EROD activity in the same cell conditions. For each gene, we chose to examine the polymorphisms that have been previously reported to be associated with altered enzymatic activity or increased cancer risk.

2. Material and methods

2.1. Study population

The study subjects were recruited between December 1992 and March 1994. Sixty-two subjects were randomly sampled from the highest exposed zones (A and B) and 59 subjects from the surrounding non-contaminated area (non-ABR), frequency matched for age, gender, and smoking, as previously described (Landi et al., 1998). The population was stratified into classes by age and gender. Using a SAS software algorithm, subjects were selected within each stratified group, according to the numbers required. Every 100th subject was selected and invited to participate. In the case of a refusal, the next subject in the sequence was selected. Informed consent was obtained from all participants and the study was reviewed and approved by the local Institutional Review Board.

2.2. Measurement of TCDD in plasma

The dioxin measurements in human plasma were performed at the Centers for Disease Control and Prevention using a high-resolution gas chromatography/mass spectrometry analysis (Patterson et al., 1987). Results were reported in parts per trillion (ppt), lipid adjusted.

Of the 121 subjects, 11 samples were inadequate for analysis and were excluded from the analyses that involved TCDD plasma levels. For 23 samples that were adequate for analysis, but had results below the detection limit (median detection limit = 2.7 ppt, range 1.4–15.7), TCDD levels were estimated by dividing the lipid-adjusted detection limit by the square root of two (Hornung and Reed, 1990).

2.3. Peripheral blood mononuclear cell culture and RNA isolation

Peripheral blood mononuclear cell culture, RNA isolation, quantitative competitive reverse transcription PCR (RT-PCR), and ethoxyresorufin deethylase (EROD) assay were performed as described in Landi et al. (2003). Briefly, lymphocytes were immediately processed after blood drawing, cryopreserved and stored at −80 °C until shipment in dry ice to the NIEHS laboratories. At NIEHS, lymphocytes were stored in liquid nitrogen. RNA was extracted immediately prior to the analyses of genes' expression. For cell culture, the lymphocytes were stimulated with phytohemagglutinin and pokeweed mitogen and incubated at 37 °C for 72 h in either the presence or absence of 10 nM TCDD.

2.4. Ethoxyresorufin deethylase assay (EROD)

The EROD assay was performed immediately after the 72 h incubation of the peripheral blood lymphocytes. Enzyme activity was assessed by measuring the fluorescence (550 nm excitation, 585 emission) produced by 10^6 peripheral blood mononuclear cells after 30 min of incubation with 50 pmol ethoxyresorufin. The reaction was stopped by the addition of methanol. A standard curve containing from 0.19 to 100 pmol resorufin/well was used for quantitation. Results were expressed as activity per milligram protein. A bicinchoninic acid assay kit was used to measure protein concentrations.

2.5. Quantitative competitive reverse transcription PCR (RT-PCR)

RT-PCR measured gene expression in total RNA obtained from mononuclear cells before and after

incubation at 37 °C. Total RNA was isolated with Trireagent (Sigma Chemical Company, St. Louis, MO) and subsequently stored at −70 °C. For each RT-PCR quantitation, from five to seven RT-PCR reactions were performed. Each reaction contained 100 ng total RNA and varying but known concentrations of a heterologous recombinant internal standard. Gene-specific primers were used for the reverse transcription. For details regarding the primer sequences, reverse transcription and amplification conditions, see Landi et al. (2003). The amplified products were separated by electrophoresis on agarose gels containing ethidium bromide. NIH Image Software was used to measure the intensities of the unknown and internal standard cDNAs in digitized images of the gels.

All *CYP1A1* and *CYP1B1* gene expression results were normalized to beta-actin expression, and results reported are as *CYP1A1* or *CYP1B1* mRNA copy number per 10^5 copies of beta-actin mRNA.

2.6. Genotype analysis

Two previously established PCR-RFLP assays were used to detect the *CYP1A1* 3801T>C (Whyatt et al., 2000) and 2455A>G (I462V) polymorphisms (Olshan et al., 2000). For the *CYP1A1* 3801T>C assay, primers were (forward) 5′ agg aag aag agg agg tag ca 3′ and (reverse) 5′ cag cac agt gat tag gag tc 3′, with 57 °C annealing. For the *CYP1A1* I462V assay, primers were (forward) 5′ gct tgc ctg tcc tct atc 3′ and (reverse) 5′ aaa gac ctc cca gcg ggt aa 3′, with 53 °C annealing.

Two previously established PCR-RFLP assays were used to detect the four *CYP1B1* SNPs associated with amino acid changes: one assay for the *CYP1B1* R48G and A119S variants, and another for the *CYP1B1* L432V and N453S variants (Mammen et al., 2003). For the *CYP1B1* R48G/A119S assay, the primers were (forward) 5′ tcc cca tcc caa tcc aag 3′ and (reverse) 5′ cgg cag ccg aaa cac ac 3′, with 61 °C annealing. For the *CYP1B1* L432V/N453S assay, the primers were (forward) 5′ gcc tgt cac tat tcc tca tgc c 3′ and (reverse) 5′ gtg agc cag gat gga gat gaa g 3′, with 57 °C annealing.

2.7. Statistical analysis

CYP1A1 and *CYP1B1* gene expression and EROD activity were log-transformed to approximate normality and analyzed using parametric statistics; geometric means and 95% confidence intervals (95% CIs) are reported. We used the Student's *t*-test for group comparisons and simple linear regression to test for trend across genotypes within SNPs. Since TCDD plasma levels were associated with EROD activity in our previous work (Landi et al., 2003), and *CYP1A1* is known to be related to EROD activity, all the analyses including EROD activity were adjusted by plasma TCDD using multiple regression analysis. Similarly, multiple regression analyses were performed to evaluate the association of plasma TCDD levels with gene expression or EROD activity by *CYP1A1* and *CYP1B1* genotypes. We have previously conducted quality control studies on the cryopreserved lymphocytes, and analyzed viability, RNA quality, and cell growth by storage length, batches of experiments, and exposure status (Landi et al., 2003). We found that post-culture viability, batch of experiment (samples were divided in 17 batches for the analyses), and percentage cell growth were associated with both TCDD plasma levels and gene expression. Thus, we adjusted all analyses of the association between TCDD plasma levels and gene expression or EROD activity for these variables. In addition, we excluded all lymphocytes with pre-cultured viability <75% from all the analyses based on uncultured cells.

For the results of inducibility ('delta-expression', calculated as gene expression in TCDD/mitogen-treated cells minus gene expression in cells treated with mitogen only), no transformation produced a suitable approximation to normality. For these data, we used non-parametric statistics and reported medians and inter-quartile ranges (IQR = 25th–75th centiles). We used the Wilcoxon (Mann–Whitney) two-sample rank sum test for group comparisons, the Spearman's rank correlation statistics to test for trend across genotypes within SNPs, and median regression models (Gould and Rogers, 1994) for the association between *CYP1A1* polymorphisms and the difference of EROD activity adjusted for plasma TCDD.

The total number of subjects varies across analyses due to missing values within each analysis.

All of the analyses reported above were performed with the use of the Stata statistical package (Stata, Version 8.0, Stata Corporation, College Station, TX).

To estimate haplotype frequencies, we used a log-linear model embedded within an expectation-maximization (EM) algorithm for phase uncertainty

(Chiano and Clayton, 1998; Mander, 2001), as implemented in Stata. The log-linear model was performed using an iterative proportional fitting algorithm (Agresti, 1992). We repeated the same analysis with an alternate method based on a gene counting EM-algorithm (Excoffier and Slatkin, 1995) as implemented in the Arlequin package, Version 2.000 (Genetics and Biometry Laboratory, University of Geneva, Switzerland). Single locus Hardy–Weinberg equilibria at *CYP1A1* and *CYP1B1* SNPs were calculated using a Markov-chain random walk algorithm (Guo and Thompson, 1992). Haplotype diversity, measuring the probability that two randomly chosen haplotypes are different in a sample, and D', measuring the deviation from random association between alleles at different loci (Lewontin, 1971), were calculated using the Arlequin 2.000 package. The *P*-value for linkage disequilibrium among SNPs at *CYP1A1* and *CYP1B1* loci were derived from a likelihood ratio test of haplotype frequencies (assuming LD versus not assuming LD) by a permutation procedure (Slatkin and Excoffier, 1996).

We analyzed the association between estimated haplotypes and the logarithm of mRNA gene expression by the Stata function 'qhapipf' (Mander, 2002). Haplotype frequencies were estimated and their regression coefficients for *CYP1B1* mRNA expression were obtained in a multiple-locus single-point additive model (SAM), which assumes additive effects for each single allele. The significance of the association between alleles and gene expression was measured by likelihood ratio test. The EM-algorithm does not allow one to assign haplotype alleles to each individual, thus mean *CYP1B1* expression for each possible pair of haplotype alleles was estimated by linear combination of the individual allelic regression coefficients. Results were expressed, after exponentiation, as geometric means and relative 95% CIs. We also repeated the same analysis without specifying the genetic model, allowing for interaction between the same variant alleles in two different chromosomes. For the analysis of the association between *CYP1B1* haplotypes and *CYP1B1* inducibility (delta-expression), we estimated the haplotype frequency counts and tested their association with tertiles of *CYP1B1* inducibility using Fisher's exact test.

All statistical tests were two-sided. A P-value < 0.05 was considered statistically significant.

2.8. Nomenclature

We used the nomenclature system for *CYP1A1* and *CYP1B1* alleles recommended by the Human Cytochrome P450 Allele Nomenclature Committee; see http://www.imm.ki.se/CYPalleles.

3. Results

3.1. Genotype and haplotype estimation

3.1.1. CYP1B1

Among the Seveso subjects evaluated for the *CYP1B1* SNPs, no significant deviation from Hardy–Weinberg equilibrium was noted. As expected (McLellan et al., 2000; Stoilov et al., 1998), the R48G and A119S variants were in complete allelic identity in this sample and are described here as a single biallelic locus. In agreement with previous studies (Bailey et al., 1998; Stoilov et al., 1998), the most frequent variant was L432V (present in 85.2% of the subjects); the least frequent was the N453S variant (in 28.7% subjects). Only three subjects (2.7%) were homozygous for the reference sequence at all *CYP1B1* SNPs, indicating the unambiguous presence of two *CYP1B1*1* alleles. A large number of subjects were heterozygous at multiple loci, making haplotype determination ambiguous. Therefore, we performed haplotype estimation among the 111 subjects with complete genotype information ($N = 222$ estimated haplotypes) (Table 1). No haplotype was estimated to carry variant alleles at all three *CYP1B1* SNPs in this sample (estimated probability for carrying more than

Table 1
Haplotype frequency estimation for *CYP1B1* gene

Nucleotide change[a]				Amino acid change	Haplotype[b]		
142	355	4326	4390		Allele	Count	%
C	G	C	A	Reference	*CYP1B1*1*	27	12.2
G	***T***	C	A	R48G; A119S	*CYP1B1*2*	69	31.1
C	G	***G***	A	L432V	*CYP1B1*3*	88	39.6
C	G	C	***G***	N453S	*CYP1B1*4*	38	17.1

[a] Nucleotide position relative to start codon.
[b] Haplotype allelic frequency estimated by the expectation-maximization (EM) algorithm using both Arlequin (Schneider, 2000) and Stata software (Mander, 2002).

Table 2
Constitutive and TCDD-induced *CYP1B1* gene expression by *CYP1B1* genotype

Polymorphism	Reference			Heterozygous			Homozygous variant			*P*-trend[a]
	n	Mean[b]	95% CI	*n*	Mean[b]	95% CI	*n*	Mean[b]	95% CI	
CYP1B1 expression (copies/10^5 copies of actin) in uncultured cells[c]										
CYP1B1 (R48G, A119S)	35	68.2	39.1–119.1	33	63.1	37.1–107.3	4	158.5	16.1–1558.3	0.64
CYP1B1 (L432V)	22	69.0	32.5–146.2	42	73.7	46.6–116.7	10	66.4	19.1–231.1	0.99
CYP1B1 (N453S)	51	78.5	51.0–120.9	21	53.8	25.2–115.1	2	116.0	0.1–106902	0.58
CYP1B1 expression (copies/10^5 copies of actin) in mitogen-treated cells										
CYP1B1 (R48G, A119S)	40	86.3	63.1–118.2	29	88.8	64.0–123.2	6	130.6	54.1–315.1	0.44
CYP1B1 (L432V)	22	119.7	76.1–188.2	42	80.1	60.2–106.6	12	91.1	51.8–160.0	0.28
CYP1B1 (N453S)	53	90.5	70.0–117.0	19	81.6	54.0–123.3	4	195.5	19.5–1961	0.43
CYP1B1 expression (copies/10^5 copies of actin) in TCDD/mitogen-treated cells										
CYP1B1 (R48G, A119S)	40	506.3	386.7–662.9	28	412.1	307.0–553.1	6	416.2	241.6–717.1	0.32
CYP1B1 (L432V)	20	372.1	259.6–533.5	43	443.2	348.4–563.8	12	692.2	436.2–1098	0.04
CYP1B1 (N453S)	52	466.1	367.8–590.7	19	430.6	309.4–599.3	4	419.0	276.7–634.5	0.68
	n	Median	IQR[e]	*n*	Median	IQR[e]	*n*	Median	IQR[e]	
Delta *CYP1-B1* expression (copies/10^5 copies of beta actin)[d]										
CYP1B1 (R48G, A119S)	39	442.8	204.4–601.3	28	320.2	163.2–488.8	6	339.6	252.6–396.5	0.08
CYP1B1 (L432V)	20	325.4	186.0–513.6	42	358.3	186.0–513.6	12	508.0	449.8–714.2	0.004
CYP1B1 (N453S)	52	427.1	213.2–515.6	18	339.6	173.3–562.0	4	136.4	−155.1–364.2	0.15

[a] Test for trend across genotype categories.
[b] Geometric mean.
[c] Samples with low viability (<75%) were excluded from the analysis.
[d] Difference in mRNA levels between lymphocytes cultured in vitro with mitogen + TCDD and lymphocytes treated with mitogen only. Results based on non-parametric analyses are reported.
[e] Inter-quartile range.

one variant per haplotype was $<5.01 \times 10^{-8}$). Haplotype diversity was $72 \pm 1.5\%$. Linkage disequilibrium among the four *CYP1B1* SNPs was complete, with a D' of -1 overall and in all expression and cell culture strata reported.

3.1.2. CYP1A1

Consistent with previous studies in Caucasians (Garte et al., 2001), the frequency of *CYP1A1* 3801T>C and *CYP1A1* I462V variants in the Seveso sample were low. Only 19 subjects (16.2%) had the 3801T>C variant. Among the seven subjects (6%) with the I462V variant, the 3801T>C variant was also present. Linkage disequilibrium among the *CYP1A1* SNPs was high, with $D' = 0.86$ overall. Given the low frequency of variant alleles and haplotype counts, we do not report the analyses on the CYP1A1 haplotypes.

3.2. Association between TCDD plasma levels and CYPs expression by genotypes and haplotypes

Overall, TCDD plasma levels were not associated with the expression of *CYP1A1* and *CYP1B1* genes and negatively associated with EROD activity in TCDD/mitogen-treated cells (Landi et al., 2003). The presence of *CYP1A1* or *CYP1B1* variant alleles did not modify the associations between TCDD plasma levels and *CYP1A1* or *CYP1B1* gene expression or between TCDD plasma levels and EROD activity (data not shown).

3.3. Association between CYPs genotypes and haplotypes and gene expression

3.3.1. CYP1B1

In uncultured cells, there was no significant difference in *CYP1B1* mRNA expression levels by *CYP1B1* genotype (Table 2). We repeated the same analysis separately in subjects from the exposed zones (A and B) and in subjects from the surrounding non-contaminated area. No major differences were present between the two groups of subjects (data not shown), but the analysis was based on small numbers. Irrespective of genotype, TCDD/mitogen treatment produced a 6.3-fold increase in *CYP1B1* expression compared with

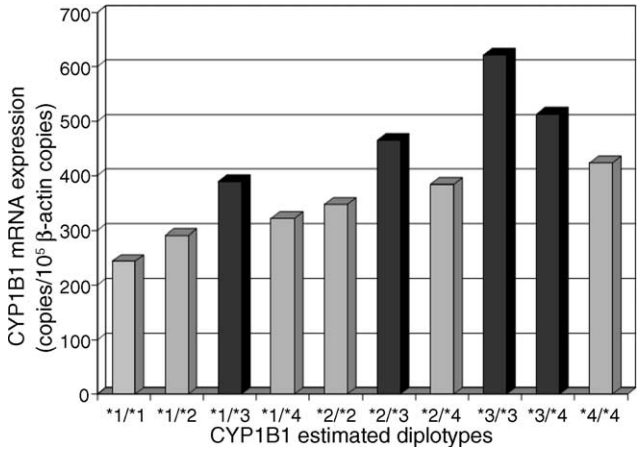

Fig. 1. Estimated *CYP1B1* diplotypes and TCDD-induced *CYP1B1* mRNA expression (geometric means) in single additive model. **1* = A119, R48, L432, N453; **2* = G48, S119, L432, N453; **3* = R48, A119, V423, N453; **4* = R48, A119, L432, S453. The darker columns indicate the estimated *CYP1B1* mRNA expression associated with one or two **3* haplotypes.

uncultured cells (mean copy number 461.1, 95% CI 388.5–547.3 versus 73.7, 95% CI 52.1–104.3, all genotypes averaged). Induction in TCDD/mitogen-treated cells was lower (2.3-fold) in the presence of a reference *CYP1B1* genotype (mean = 68.9, 95% CI 0.1–575.5 and 30.1, 95% CI 0.5–1827.8, in TCDD/mitogen-treated lymphocytes and uncultured cells, respectively) than in subjects with at least one *CYP1B1* variant (mean = 475.6, 95% CI 400.4–565.1 and mean = 73.3, 95% CI 50.5–106.6 in TCDD/mitogen-treated lymphocytes and uncultured cells, respectively; 6.5-fold). In TCDD/mitogen-treated lymphocytes, subjects who carried at least one V432 variant (suggestive of a *CYP1B1*3* allele) showed higher *CYP1B1* mRNA expression in comparison to subjects with a L432 genotype ($P = 0.04$, test for trend across L432V genotype categories, Table 2).

In order to distinguish the effect of in vitro TCDD treatment from that of the mitogen, we calculated the difference between *CYP1B1* gene expression in TCDD/mitogen-treated cells and *CYP1B1* expression in mitogen only-treated cells (delta-expression).

There was a statistically significant positive trend in inducibility when we compared subjects with the *CYP1B1* L432 genotype and subjects either heterozygous or homozygous for the V432 variant ($P = 0.004$, test for trend, Table 2).

The single-point additive linear regression model based on haplotype estimation confirmed the *CYP1B1*3* allele was the only variant allele significantly associated with gene expression. When the three variant alleles (*CYP1B1*2*, *CYP1B1*3*, and *CYP1B1*4*) were considered in the same regression model, only *CYP1B1*3* was significantly associated with the *CYP1B1* mRNA levels (regression coefficient = 0.48, $P = 0.03$, likelihood ratio test). The regression coefficients obtained from the single-point additive model (SAM), were used to estimate the mean expression of *CYP1B1* mRNA in subjects carrying the possible combinations of variant allele pairs or diplotypes (Fig. 1). The lowest gene expression was estimated for the subjects carrying two *CYP1B1*1* alleles (mean = 243.9; CI 95% 88.9–669.0), while the highest expression was in the subjects carrying two *CYP1B1*3* alleles (mean = 621.7, 95% CI = 451.4–856.2). We repeated the same analysis adding the interaction terms for each variant pair on the two chromosomes (data not shown). No major differences were observed between the two models (P-value = 0.78, likelihood ratio test for the differences between the two models).

No statistically significant association was found between any *CYP1B1* haplotype and *CYP1B1* 'delta-expression', calculated as gene expression in TCDD/mitogen-treated cells minus gene expression in cells treated with mitogen only ($P = 0.78$, Fisher's exact). Failure to observe an association may be in part due to low statistical power, since only non-parametric

analysis based on tertiles of 'delta-expression' could be performed.

Given the association of the *CYP1B1*3* variant allele with expression, we questioned whether *CYP1B1*3* subjects developed more diseases following the accident than subjects with the reference allele or other *CYP1B1* variant alleles. We examined collected data on all health conditions reported by the subjects from the moment of the accident (1976) to the study period (1992–1994). Data was available for several health conditions, including allergic, gastrointestinal, infectious, endocrine, and respiratory diseases, in 115 individuals. Subjects with cancer or chloracne were not included in this study. No major differences for any of the conditions were observed by *CYP1B1* alleles, with the exception of an increased frequency of eczema ($n = 8$, $P = 0.05$) and urticaria ($n = 2$, $P = 0.02$) in subjects with the *CYP1B1*3* variant allele.

3.3.2. CYP1A1 and EROD activity

CYP1A1 mRNA levels and EROD activity were strongly induced (8.8-fold and 13.1-fold, respectively, $P < 0.001$) when 10 nM TCDD was added to the mitogen-stimulated cells (Landi et al., 2003). In TCDD/mitogen-treated cells, *CYP1A1* gene expression was almost 2-fold higher in subjects with a heterozygous *CYP1A1* I462V genotype ($n = 4$, geometric mean = 618.2) than subjects with the I462 (reference) genotype ($n = 74$, geometric mean = 361.2), but the difference was based on the few subjects who also carried the *CYP1A1* 3801T>C SNP, and was not statistically significant ($P = 0.09$). Similarly, EROD activity did not vary between subjects with the reference *CYP1A1* genotype and subjects with at least one *CYP1A1* variant. When subjects were categorized as carrying reference alleles, *CYP1A1* 3801T>C only or both *CYP1A1* 3801T>C and I462V variant alleles, subjects who carried both variant alleles had the highest *CYP1A1* mRNA levels, but the difference among the three groups was not statistically significant ($P = 0.20$, test for trend). No significant difference in *CYP1A1* gene expression was observed between subjects who carried both *CYP1A1* and *CYP1B1* variant alleles in comparison with subjects who carried reference genotypes for both *CYP1A1* and *CYP1B1* genes. Similarly, no significant differences in *CYP1A1* expression or EROD activity were observed when subjects were stratified by *CYP1B1* genotypes (data not shown).

4. Discussion

To our knowledge, this is the first study to examine the relationship between cytochrome P450 genes' haplotypes and either *CYP1A1* and *CYP1B1* gene expression or EROD activity in human lymphocytes.

The presence of variants in the *CYP1A1* and *CYP1B1* genes did not modify the lack of association between TCDD plasma levels and *CYP* mRNA expression or EROD activity in lymphocytes obtained from subjects randomly selected from the exposed and surrounding non-contaminated area of Seveso, Italy. However, subjects heterozygous or homozygous for the *CYP1B1* V432 variant (suggestive of the common *CYP1B1*3* allele) showed higher TCDD-induced expression of *CYP1B1* than subjects with the L432 homozygous genotype (Table 2). This effect was also seen when considering the delta-expression of *CYP1B1* mRNA levels in lymphocytes from individuals with the *CYP1B1* V432 variant (Table 2).

We performed haplotype analysis to verify whether there were effects on gene expression due to haplotype alleles that were undetected by genotype analysis. Haplotype analysis allowed us to estimate: (a) the likelihood of carrying recombinant alleles, and (b) the association between each *CYP1B1* allele or their combination and *CYP1B1* gene expression.

(a) We observed that only two subjects (with *CYP1B1* expression data) were homozygous for *CYP1B1*1*, the reference allele, while many subjects were heterozygous for several variant allele combinations. The probability of additional recombinant haplotypes (*CYP1B**5, *CYP1B*6* or *CYP1B**7 alleles; Aklillu et al., 2002) or other recombined haplotypes) was estimated to be close to zero, and there was complete linkage disequilibrium ($D' = -1$) among the variant loci.

(b) The single-point additive model confirmed a significant association between the *CYP1B1*3* allele and *CYP1B1* RNA levels, consistent with the effect of the V432 genotype observed in our genotype analysis. Thus, we conclude that the SNP that causes the L432V amino acid change is the functional SNP, at least within the limits of our study. In addition, the analysis of possible combinations of the same alleles in two different chromosomes (Fig. 1), showed that the homozygous

*CYP1B1*3* combination was associated with the highest *CYP1B1* expression, while the homozygous *CYP1B1*1* combination had the lowest expression. We were unable to detect evidence for interaction between different alleles at the same locus. Thus, the effect of *CYP1B1*3* allele on *CYP1B1* gene expression appears to follow an additive or codominant model. These results need to be replicated in larger studies, since our analysis was limited for the following reasons: haplotypes were imputed from observed genotype data rather than measured directly, the studied SNPs were located within coding regions, were in complete linkage disequilibrium with each other and were contained within a single region of linkage disequilibrium (i.e., no recombinant alleles were observed).

The *CYP1B1*3* allele is the most common allele in populations of European and African descent (Mammen et al., 2003) and may likely be the ancestral *CYP1B1* haplotype. Our observation that *CYP1B1*3* alleles are associated with higher mRNA inducibility following in vitro TCDD treatment is interesting. The SNP that causes the L432V amino acid change could affect mRNA stability, or be in linkage disequilibrium with other variants in *CYP1B1* transcriptional regulatory sequences such as response elements or those regulating RNA degradation (Cartegni et al., 2002). For example, there are many other SNPs in the *CYP1B1* non-coding and flanking regions that might affect expression. In previous biochemical studies, the CYP1B1.3 protein had similar activity to other common protein variants (Li et al., 2000; Mammen et al., 2003; Shimada et al., 1999). However, analysis of a *CYP1B1* enzyme with the 432V substitution showed increased apparent *K*(m) after coexpression in *E. coli* with NAPDH-cytochrome P450 reductase (Shimada et al., 2003). In addition, the CYP1B1.6 and CYP1B1.7 proteins, which both include the V432 substitution (in addition to other amino acid changes), exhibited altered kinetics with significantly increased apparent *K*(m) and lowered *V*(max) values for both the 2- and 4-hydroxylation of 17 beta-estradiol when evaluated after heterologous expression in *S. cerevisiae* (Aklillu et al., 2002). Further functional studies are necessary to explore the relationship between the *CYP1B1*3* allele, regulation of inducible *CYP1B1* transcription and CYP1B1 enzymatic activity.

In uncultured lymphocytes, *CYP1B1* expression was not associated with TCDD exposure (Landi et al., 2003), either in the presence of a reference or variant *CYP1B1* genotype. The enhanced effect on gene expression of TCDD in cell culture in comparison with circulating TCDD could be due to the relatively low dose of plasma TCDD in these subjects after almost 20 years from the original exposure, while the dose of in vitro TCDD and time of culture we used are known to be optimal for maximal induction in vitro (Spencer et al., 1999). However, one might speculate that the *CYP1B1*3* allele could have modified the effect of dioxin when subjects were acutely exposed to very high levels of dioxin at the time of the accident. To verify this hypothesis, we examined the distribution of the diseases developed by the exposed subjects in the 20 years after the accident, stratified by *CYP1B1* alleles. Although based on very small number of subjects, there was a suggestive increase of allergic skin diseases (urticaria and eczema) in subjects carrying the *CYP1B1*3* allele. This finding needs to be verified in larger studies.

Previous work in lymphocytes (Masten et al., 1998; Spencer et al., 1999) and epithelial cells (Eltom et al., 1998; Larsen et al., 1998) have shown constitutive levels of *CYP1B1* mRNA are high compared to those of *CYP1A1*. Consequently, we measured *CYP1A1* expression in mitogen-stimulated cells only. As expected in Caucasians (Garte et al., 2001), the frequency of *CYP1A1* variants was low. Even though based on small numbers, subjects heterozygous for the *CYP1A1* I462V variant, who also had the *CYP1A1* 3801T>C variant, had a higher *CYP1A1* expression and *CYP1A1* inducibility than subjects with the reference genotype.

No differences were observed in EROD activity by variants of *CYP1A1* or *CYP1B1* genes. Previous studies have shown that *CYP1A1* variants were associated with increased enzyme activity (Crofts et al., 1994; Kiyohara et al., 1998; Landi et al., 1994; Puga et al., 1997). However, in other studies involving either phenotyping or in vitro expression, there was no correlation between *CYP1A1* activity and *CYP1A1* variants (Jacquet et al., 1996; Zhang et al., 1996). Larger studies are necessary to further explore the association between *CYP1A1* gene and related RNA and functional activity, since *CYP1A1* variant alleles are infrequent in Caucasians (Garte et al., 2001).

In conclusion, polymorphisms in the *CYP1B1* or *CYP1A1* genes did not modify the lack of association between TCDD plasma levels and *CYP1A1* or *CYP1B1* gene expression in lymphocytes from Seveso subjects, approximately 20 years after the accident. However, in vitro TCDD treatment of cultured lymphocytes strongly induced both *CYP1A1* and *CYP1B1* expression. The *CYP1B1*3* allele modified *CYP1B1* expression in an additive fashion.

This study presents evidence of inter-individual variability in cytochrome P450 induction due to genetic variation, and may help in identifying subjects with variable responsiveness to TCDD and potentially increased cancer risk.

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References

Agresti, A., 1992. Modelling patterns of agreement and disagreement. Stat. Meth. Med. Res. 1, 201–218.

Aklillu, E., Oscarson, M., Hidestrand, M., Leidvik, B., Otter, C., Ingelman-Sundberg, M., 2002. Functional analysis of six different polymorphic CYP1B1 enzyme variants found in an Ethiopian population. Mol. Pharmacol. 61, 586–594.

Bailey, L.R., Roodi, N., Dupont, W.D., Parl, F.F., 1998. Association of cytochrome P450 1B1 (cyp1b1) polymorphism with steroid receptor status in breast cancer. Cancer Res. 58, 5038–5041.

Bejjani, B.A., Stockton, D.W., Lewis, R.A., Tomey, K.F., Dueker, D.K., Jabak, M., et al., 2000. Multiple CYP1B1 mutations and incomplete penetrance in an inbred population segregating primary congenital glaucoma suggest frequent de novo events and a dominant modifier locus. Hum. Mol. Genet. 9, 367–374.

Bertazzi, P.A., Consonni, D., Bachetti, S., Rubagotti, M., Baccarelli, A., Zocchetti, C., et al., 2001. Health effects of dioxin exposure: a 20-year mortality study. Am. J. Epidemiol. 153, 1031–1044.

Bertazzi, P.A., Pesatori, A.C., Consonni, D., Tironi, A., Landi, M.T., Zocchetti, C., 1993. Cancer incidence in a population accidentally exposed to 2,3,7,8-tetrachlorodibenzo-*para*-dioxin. Epidemiology 4, 398–406.

Birnbaum, L.S., Tuomisto, J., 2000. Non-carcinogenic effects of TCDD in animals. Food Addit. Contam. 17, 275–288.

Cartegni, L., Chew, S.L., Krainer, A.R., 2002. Listening to silence and understanding nonsense: exonic mutations that affect splicing. Nat. Rev. Genet. 3, 285–298.

Cascorbi, I., Brockmoller, J., Roots, I., 1996. A C4887A polymorphism in exon 7 of human CYP1A1: population frequency, mutation linkages, and impact on lung cancer susceptibility. Cancer Res. 56, 4965–4969.

Chiano, M.N., Clayton, D.G., 1998. Fine genetic mapping using haplotype analysis and the missing data problem. Ann. Hum. Genet. 62 (Pt 1), 55–60.

Crofts, F., Taioli, E., Trachman, J., Cosma, G.N., Currie, D., Toniolo, P., et al., 1994. Functional significance of different human CYP1A1 genotypes. Carcinogenesis 15, 2961–2963.

Dragan, Y.P., Schrenk, D., 2000. Animal studies addressing the carcinogenicity of TCDD (or related compounds) with an emphasis on tumor promotion. Food Addit. Contam. 17, 289–302.

Eltom, S.E., Larsen, M.C., Jefcoate, C.R., 1998. Expression of CYP1B1 but not CYP1A1 by primary cultured human mammary stromal fibroblasts constitutively and in response to dioxin exposure: role of the AH receptor. Carcinogenesis 19, 1437–1444.

Excoffier, L., Slatkin, M., 1995. Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol. Biol. Evol. 12, 921–927.

Fritsche, E., Bruning, T., Jonkmanns, C., Ko, Y., Bolt, H.M., Abel, J., 1999. Detection of cytochrome p450 1B1 BFR I polymorphism: genotype distribution in healthy German individuals and in patients with colorectal carcinoma. Pharmacogenetics 9, 405–408.

Garte, S., Gaspari, L., Alexandrie, A.K., Ambrosone, C., Autrup, H., Autrup, J.L., et al., 2001. Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol. Biomark. Prev. 10, 1239–1248.

Goodman, M.T., McDuffie, K., Kolonel, L.N., Terada, K., Donlon, T.A., Wilkens, L.R., et al., 2001. Case-control study of ovarian cancer and polymorphisms in genes involved in catecholestrogen formation and metabolism. Cancer Epidemiol. Biomark. Prev. 10, 209–216.

Gould, W., Rogers, W.H., 1994. Quantile regression as an alternative to robust regression. In: Proceedings of the Statistical Computing Section, American Statistical Association, Alexandria, VA.

Guo, S.W., Thompson, E.A., 1992. Performing the exact test of Hardy–Weinberg proportion for multiple alleles. Biometrics 48, 361–372.

Hoffman, E.C., Reyes, H., Chu, F.F., Sander, F., Conley, L.H., Brooks, B.A., et al., 1991. Cloning of a factor required for activity of the AH (dioxin) receptor. Science 252, 954–958.

Hornung, R.W., Reed, L.D., 1990. Estimation of average concentration in the presence of non-detectable values. Appl. Occup. Environ. Hyg. 5, 48–51.

Houlston, R.S., 2000. CYP1A1 polymorphisms and lung cancer risk: a meta-analysis. Pharmacogenetics 10, 105–114.

Hung, R.J., Boffetta, P., Brockmoller, J., Butkiewicz, D., Cascorbi, I., Clapper, M.L., et al., 2003. CYP1A1 and GSTM1 genetic polymorphisms and lung cancer risk in caucasian non-smokers: a pooled analysis. Carcinogenesis 24, 875–882.

Ingelman-Sundberg, M., 2002. Polymorphism of cytochrome p450 and xenobiotic toxicity. Toxicology 181–182, 447–452.

Jacquet, M., Lambert, V., Baudoux, E., Muller, M., Kremers, P., Gielen, J., 1996. Correlation between p450 CYP1A1 inducibility, mspi genotype and lung cancer incidence. Eur. J. Cancer A 32, 1701–1706.

Jiang, B.H., Rue, E., Wang, G.L., Roe, R., Semenza, G.L., 1996. Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. J. Biol. Chem. 271, 17771–17778.

Kiyohara, C., Nakanishi, Y., Inutsuka, S., Takayama, K., Hara, N., Motohiro, A., et al., 1998. The relationship between CYP1A1 aryl hydrocarbon hydroxylase activity and lung cancer in a Japanese population. Pharmacogenetics 8, 315–323.

Ko, Y., Abel, J., Harth, V., Brode, P., Antony, C., Donat, S., et al., 2001. Association of CYP1B1 codon 432 mutant allele in head and neck squamous cell cancer is reflected by somatic mutations of p53 in tumor tissue. Cancer Res. 61, 4398–4404.

Landi, M.T., Bertazzi, P.A., Baccarelli, A., Consonni, D., Masten, S., Lucier, G., et al., 2003. TCDD-mediated alterations in the AHR-dependent pathway in Seveso, Italy, 20 years after the accident. Carcinogenesis 24, 673–680.

Landi, M.T., Bertazzi, P.A., Shields, P.G., Clark, G., Lucier, G.W., Garte, S.J., et al., 1994. Association between cyp1a1 genotype, mRNA expression and enzymatic activity in humans. Pharmacogenetics 4, 242–246.

Landi, M.T., Consonni, D., Patterson Jr., D.G., Needham, L.L., Lucier, G., Brambilla, P., et al., 1998. 2,3,7,8-Tetrachlorodibenzo-*p*-dioxin plasma levels in Seveso 20 years after the accident. Environ. Health Perspect. 106, 273–277.

Landi, M.T., Needham, L.L., Lucier, G., Mocarelli, P., Bertazzi, P.A., Caporaso, N., 1997. Concentrations of dioxin 20 years after Seveso. Lancet 349, 1811.

Larsen, M.C., Angus, W.G., Brake, P.B., Eltom, S.E., Sukow, K.A., Jefcoate, C.R., 1998. Characterization of CYP1B1 and CYP1A1 expression in human mammary epithelial cells: role of the aryl hydrocarbon receptor in polycyclic aromatic hydrocarbon metabolism. Cancer Res. 58, 2366–2374.

Le Marchand, L., Guo, C., Benhamou, S., Bouchardy, C., Cascorbi, I., Clapper, M.L., et al., 2003. Pooled analysis of the CYP1A1 exon 7 polymorphism and lung cancer (United States). Cancer Causes Control. 14, 339–346.

Lewontin, R.C., 1971. The effect of genetic linkage on the mean fitness of a population. Proc. Natl. Acad. Sci. U.S.A. 68, 984–986.

Li, D.N., Seidel, A., Pritchard, M.P., Wolf, C.R., Friedberg, T., 2000. Polymorphisms in p450 cyp1b1 affect the conversion of estradiol to the potentially carcinogenic metabolite 4-hydroxyestradiol. Pharmacogenetics 10, 343–353.

Mammen, J.S., Pittman, G.S., Li, Y., Abou-Zahr, F., Bejjani, B.A., Bell, D.A., et al., 2003. Single amino acid mutations, but not common polymorphisms, decrease the activity of CYP1B1 against (−)benzo[a]pyrene-7r-trans-7,8-dihydrodiol. Carcinogenesis 24, 1247–1255.

Mander, A.P., 2001. Haplotype analysis in population-based association studies. Stata J. 1, 58–75.

Mander, A.P., 2002. Analysis of quantitative traits using regression and log-linear modeling when phase is unknown. Stata J. 2, 65–70.

Masten, S.A., Grassman, J.A., Miller, C.R., Spencer, D.L., Walker, N.J., Jung, D., et al., 1998. Population-based studies of dioxin responsiveness: individual variation in cyp1a1 levels and relationship to dioxin body burden. Organohalogen Comp. 37, 13–16.

McLellan, R.A., Oscarson, M., Hidestrand, M., Leidvik, B., Jonsson, E., Otter, C., et al., 2000. Characterization and functional analysis of two common human cytochrome p450 1B1 variants. Arch. Biochem. Biophys. 378, 175–181.

Olshan, A.F., Weissler, M.C., Watson, M.A., Bell, D.A., 2000. GSTM1, GSTT1, GSTP1, CYP1A1, and NAT1 polymorphisms, tobacco use, and the risk of head and neck cancer. Cancer Epidemiol. Biomark. Prev. 9, 185–191.

Patterson Jr., D.G., Hampton, L., Lapeza Jr., C.R., Belser, W.T., Green, V., Alexander, L., et al., 1987. High-resolution gas chromatographic/high-resolution mass spectrometric analysis of human serum on a whole-weight and lipid basis for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. Anal. Chem. 59, 2000–2005.

Pesatori, A.C., Tironi, A., Consonni, D., Baccarelli, A., Rubagotti, M., Bachetti, S., et al., 1999. Cancer incidence in the Seveso population, 1977–1991. Organohalogen Comp. 44, 411–412.

Puga, A., Barnes, S.J., Dalton, T.P., Chang, C., Knudsen, E.S., Maier, M.A., 2000. Aromatic hydrocarbon receptor interaction with the retinoblastoma protein potentiates repression of e2f-dependent transcription and cell cycle arrest. J. Biol. Chem. 275, 2943–2950.

Puga, A., Nebert, D.W., McKinnon, R.A., Menon, A.G., 1997. Genetic polymorphisms in human drug-metabolizing enzymes: potential uses of reverse genetics to identify genes of toxicological relevance. Crit. Rev. Toxicol. 27, 199–222.

Safe, S.H., 1986. Comparative toxicology and mechanism of action of polychlorinated dibenzo-*p*-dioxins and dibenzofurans. Annu. Rev. Pharmacol. Toxicol. 26, 371–399.

Sasaki, M., Tanaka, Y., Kaneuchi, M., Sakuragi, N., Dahiya, R., 2003. CYP1B1 gene polymorphisms have higher risk for endometrial cancer, and positive correlations with estrogen receptor alpha and estrogen receptor beta expressions. Cancer Res. 63, 3913–3918.

Schneider, S., 2000. Arlequin Ver. 2.000: A Software for Population Genetics Data Analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Shimada, T., Sugie, A., Shindo, M., Nakajima, T., Azuma, E., Hashimoto, M., et al., 2003. Tissue-specific induction of cytochromes P450 1A1 and 1b1 by polycyclic aromatic hydrocarbons and polychlorinated biphenyls in engineered c57bl/6j mice of arylhydrocarbon receptor gene. Toxicol. Appl. Pharmacol. 187, 1–10.

Shimada, T., Watanabe, J., Kawajiri, K., Sutter, T.R., Guengerich, F.P., Gillam, E.M., et al., 1999. Catalytic properties of polymorphic human cytochrome p450 1B1 variants. Carcinogenesis 20, 1607–1613.

Slatkin, M., Excoffier, L., 1996. Testing for linkage disequilibrium in genotypic data using the expectation-maximization algorithm. Heredity 76, 377–383.

Spencer, D.L., Masten, S.A., Lanier, K.M., Yang, X., Grassman, J.A., Miller, C.R., et al., 1999. Quantitative analysis of constitutive and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin-induced cytochrome P450 1B1 expression in human lymphocytes. Cancer Epidemiol. Biomark. Prev. 8, 139–146.

Spurr, N.K., Gough, A.C., Stevenson, K., Wolf, C.R., 1987. Msp-1 polymorphism detected with a cdna probe for the p-450 1 family on chromosome 15. Nucl. Acids Res. 15, 5901.

Stoilov, I., Akarsu, A.N., Alozie, I., Child, A., Barsoum-Homsy, M., Turacli, M.E., et al., 1998. Sequence analysis and homology modeling suggest that primary congenital glaucoma on 2p21 results from mutations disrupting either the hinge region or the conserved core structures of cytochrome p4501b1. Am. J. Hum. Genet. 62, 573–584.

Taioli, E., Gaspari, L., Benhamou, S., Boffetta, P., Brockmoller, J., Butkiewicz, D., et al., 2003. Polymorphisms in CYP1A1, GSTM1, GSTT1 and lung cancer below the age of 45 years. Int. J. Epidemiol. 32, 60–63.

Tanaka, Y., Sasaki, M., Kaneuchi, M., Shiina, H., Igawa, M., Dahiya, R., 2002. Polymorphisms of the CYP1B1 gene have higher risk for prostate cancer. Biochem. Biophys. Res. Commun. 296, 820–826.

Watanabe, J., Shimada, T., Gillam, E.M., Ikuta, T., Suemasu, K., Higashi, Y., et al., 2000. Association of CYP1B1 genetic polymorphism with incidence to breast and lung cancer. Pharmacogenetics 10, 25–33.

Whitlock, J.P., 1999. Induction of cytochrome P4501A1. Annu. Rev. Pharmacol. Toxicol. 39, 103–125.

Whyatt, R.M., Perera, F.P., Jedrychowski, W., Santella, R.M., Garte, S., Bell, D.A., 2000. Association between polycyclic aromatic hydrocarbon–DNA adduct levels in maternal and newborn white blood cells and glutathione S-transferase P1 and CYP1A1 polymorphisms. Cancer Epidemiol. Biomark. Prev. 9, 207–212.

Zhang, Z.Y., Fasco, M.J., Huang, L., Guengerich, F.P., Kaminsky, L.S., 1996. Characterization of purified human recombinant cytochrome P4501A1-ile462 and -val462: assessment of a role for the rare allele in carcinogenesis. Cancer Res. 56, 3926–3933.